STUDY OF DNA DAMAGE INDUCED BY LOW AND HIGH LINEAR ENERGY TRANSFER (LET) RADIATION

ABSTRACT

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SUBMITTED
IN PARTIAL FULFILMENT OF THE REQUIREMENT
OF THE DEGREE OF
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IN
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NORTH-EASTERN HILL UNIVERSITY
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Biological effects of radiation emancipate from the interaction of radiation with matter comprising all levels of biological organization. The interaction causes subtle and obvious "changes" in various components of cells, tissues and organism. The effected living system usually "responds" to the "changes". The living system is normally endowed with ways and means of variable magnitude and types to counter such "changes". Consequently and depending on the quality and quantity of the changes, some such changes are reverted or repaired. The remaining component or "changes" are likely to effect the process of life and are commonly referred to as "damages". The outcome of these intricately balanced and, to a great extent not clearly understood, processes culminate into cancer, heritable changes and cell death.

The damages include a variety of changes in the genetic material, which, among others, includes base changes, mutations, strand breaks and transformation. Radiation also induces and influences the process of programmed cell death. Consequences of these changes culminate into genomic instability. These events and their molecular mechanisms are only partly understood in case of low-LET radiation. On the other hand, the situation for high-LET radiation remains far from being clear. A proper assessment and evaluation of biological effects of low- and high-LET radiation is therefore, highly relevant. It is not only important in fundamental understanding of cellular response to effects of radiation, but also has applied potentials.

The search for an effective way of applying radiation to human cancer radiotherapy has been probably one of the main objective of radiobiologists. Its obvious importance has led to extensive radiobiological studies. One universal aim and interest in radiobiology research is to understand the interaction and mechanisms of action of ionizing radiation and its effects on biological systems. It may be of low linear energy transfer (LET) radiation i.e., sparsely ionizing radiations, such as X-rays, gamma rays, electrons, ultra soft X-rays or high-LET radiation i.e., densely ionizing like alpha particles, protons, neutrons etc. and other heavier particles produced by certain types of high energy accelerators. The knowledge of this is important in medicinal application such as radiotherapy and radio-diagnostics, and also for radiation protection on earth and in the space environment.

Ever since the elucidation of structure of deoxyribonucleic acid (DNA) molecule in the early 1950's by Watson and Crick, studies related to understanding and manipulation of DNA has grown by leaps and bound in various branches of biological sciences. The focus has not been different in radiobiological investigations too. Because of the universal acceptance of DNA as the genetic material, it is considered to be a critical target for damage induced by radiation in cellular systems. The damages inflicted upon by radiation

are known to cause the consequent biological effect. Apoptosis, transformation and carcinogenesis via mutation and reproductive cell death are closely related to molecular damages in the DNA. The DNA damages have been studied experimentally and theoretically employing several approaches. Consequently, several models have been established in order to explain the biological observations. At molecular level, the studies involved different endpoints like cell survival, chromosomal aberration, DNA rejoining, mutation and DNA strand breaks both single strand breaks (SSB) and double strand breaks (DSB). Yet variation in results at different conditions defies working out a firm stand in explaining the mechanism. Most of the existing studies on DNA damage give quantitative insight such as, the yields of such damages and their dependence on radiation quality. The knowledge of the quality and nature of DNA damage is limited. Interaction of radiation with a matter is random. The interaction elicits changes in the target site, which may alter the normal course of cellular metabolism, their consequential repair and expression of the effects.

One general view is that it may be due to the differential repair capacity that each living cell possesses to repair damages particularly inflicted by radiation. This has an important implication since radiosensitivity of a cell is mostly defined depending on the ability of a cell to repair the wide spectrum of DNA damages. An increasing body of experimental evidences has accumulated indicating that distribution of the DNA radiation damage and the complexity and fluidity of the nuclear organization can affect repair. This has led to an indication that the structural organization of DNA or chromatin compactness determines the radiosensitivity of cells. Well within this line of observation, experimental reports also suggest that structural organization of DNA may not be the only factor influencing the radiosensitivity. Thus, because of such diverse observations, it is not surprising that a single concept has so far not emerged in defining radiosensitivity of a cell. The molecular basis of the variable inherent radiosensitivity and genomic instability, therefore, remains enigmatic. In this context, understanding the nature of the initial lesion to its DNA and its link to the eventual expression of biological damage is of utmost importance.

In the light of these information this work envisages to study the DNA damage induced by low- and high-LET radiation with the aim of contributing in the understanding of the molecular consequences of radiation induced DNA damages.

The study has been separated into two main sections. In the first section, a system was selected where the effect of radiation on DNA damage in non-cellular condition could be clearly observed at molecular level. For this reason, naked plasmid and bacterial genomic DNA were selected. The study attempts to understand the quality and nature of

DNA damage induced by low- and high-LET radiations.

In the latter section, the investigation covers study on effect of radiation on DNA and other components, and their response in cellular condition. Mammalian cells *in vitro* and *ex vivo* was selected for this purpose. The study attempts to describe and understand the radiation induced DNA damage in relation to the biological endpoints that is measured.

The study brings out the following main points:

* Low-LET ^{60}Co γ-radiation causes predominantly DNA SSB than DSB in pMTa4 plasmid. Since plasmid DNA was irradiated in aqueous solution, $^{\bullet}$OH seems to be the major determinant in the production of SSB.

* The formation of defined extra fragments from irradiated pMTa4 DNA by *Hae II* and *Nci I* (but not by others 7 RE that were used in the investigation) suggest a non-random manifestation of effect by radiation.

* Within the conditions and parameter studied it suggest that GC-rich nucleotides were being more affected or chemically modified upon exposure to ^{60}Co γ-radiation. It may be speculated that non-GC-motif were not affected by ^{60}Co γ-radiation due to which it did not afford any resistance to certain RE (*Acc I, Bgl I, Bgl II, Dra I, Hinf I, Ksp I* and *Pvu II*).

* Unlike in low-LET, high-LET ^{7}Li particle radiation induced more DSB than SSB in pMTa4. High-LET radiation lead to a denser deposition of ionization than γ-rays and this could explain the increase in the yield of DSB.

* Even though high-LET ^{7}Li particle radiation caused more DSB than SSB the nature of damage on pMTa4, as revealed by RE approach, appeared to be similar to that observed for low-LET ^{60}Co γ-radiation. RE fragmentation analysis indicates that, though the extent and impact of the damage induced by low- and high-LET may differ, the molecular basis of damage may likely follow a similar mechanism.

* The study suggests the likely possibility that radiomodified GC nucleotides would form important premutagenic lesions. This indication also points that clusters of GC in the DNA molecule might very likely form hotspots for radiation induced damages.

* While further detailed investigation would be required, it opens up the likely possibility that inherent radiosensitivity and genome instability may be at least partly determined by the GC-richness of nucleotide sequence in the DNA.

* Since in majority of eukaryotic genomes, especially human genome, there exists "CpG islands", our results suggest a likely possibility that "genes" are more radiosensitive than "non-gene" components of a genome.

* RE can be used in the partial characterization of radiation induced specific nucleotide damage in small defined DNA molecules. This approach may also be applicable in study of damages induced by other agents than radiation.

* RE approach does not reveal any apparent modification induced by radiation in *E. coli* genomic DNA. Due to the large number of undefined fragments produced as compared to the plasmid (pMTa4), the qualitative fragmentation analysis was not revealing. This makes RE approach and analysis by agarose gel electrophoresis non-sensitive for studying specific nucleotide damage in a large and highly complex DNA molecule.

* The alkaline comet assay was able to detect ^{137}Cs γ-radiation induced SSB in human kidney T1 cells. Under these conditions, SSB favored the relaxation or decondensation of chromatin.

* SSB, however, got partially repaired or rejoined during repair permissive conditions (20-min incubation at 37 °C). Under these conditions, repair or rejoining of SSB appeared to allow the chromatin reorganize its structural configuration favoring a re-condensation.

* Using a novel immuno-blot assay (Slot and Western blot), a general inhibition of poly-ADP-ribosylation (PAR) in *ex vivo* mouse spleen cells upon ^{60}Co γ-irradiation was observed.

* The PAR of total cellular protein was inhibited initially at 1 Gy after which the level increased gradually reaching the control level. For histone proteins, the inhibition was observed up to 2-4 Gy.

* Under identical experimental conditions maximum induction of apoptosis or programmed cell death was measured at about 2 Gy in *ex vivo* mouse spleen cells upon ^{60}Co γ-irradiation. It is suggested that reduction in the level of PAR of nuclear proteins occur during the initiation of radiation induced apoptosis.

* The lowering of PAR can be due to the loss of poly-ADP-ribose polymerase (PARP) activity. The results from *ex vivo* mouse spleen cells thus, support the theory of PARP cleavage by caspases during the execution phase of apoptosis. It can be inferred that PAR degrading enzymes such as, poly-ADP-ribose glycohydrolase may also be inactivated by ^{60}Co γ-radiation.

* The lowering of PAR may be proposed as a biomarker during early stages of radiation-induced apoptosis. Thus, the immuno-blot assay of PAR can potentially be used as a predictive assay for monitoring progression of radiotherapy.

* The study with ^{11}B charged particle on three cell lines (T1, R1H and HG) *in vitro* led to loss or inactivation of acetylcholine esterase enzyme as well as cell death. These cell membrane related biochemical parameters suggest that the accelerated charged ^{11}B particle effected cell membrane variantly in different cell types *in vitro*. Further investigations are necessary to see if these changes were only physiological or extended to molecular level.

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